

### Practical 8 - Broad bean root tip squash



This practical focuses on setting up and manipulating apparatus and making and recording observations. Further skills can be developed using additional information after the practical has been completed.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Experience simple techniques to observe mitosis in cells in the root tip of broad beans
- Further your knowledge about mitosis
- Interpret and record observations

#### Safety Information

	You should wear eye protection throughout this practical.
	Acetic orcein is <b>corrosive</b> . Avoid contact with eyes or skin. It will stain skin and clothes.

#### Background information

- The role of chromosomes is to store information in the DNA coding and be able to replicate by cell division.
- Mitosis is a type of cell division found in somatic cells, that produces diploid cells.
- Chromosomes are only visible when the cell is dividing. They can be made more visible using appropriate staining techniques.
- During mitosis each chromosome separates into two chromatids.
- Each chromatid is pulled to opposite ends of the spindle prior to the cell dividing into two.
- The different stages of cell division are called interphase, prophase, metaphase, anaphase, telophase and back to interphase and are characterised by the position of the chromatids.
- During interphase the DNA of the chromatids replicates so that at prophase, whole chromosomes are visible once more.
- Mitosis can be observed in the meristematic tissues found in the apical meristem of actively growing roots of plants such as broad bean.

You will investigate how root tip tissue can be prepared and stained so that chromosomes in cells undergoing mitosis can be viewed through a microscope. You will then identify the various stages of cell division.

- Read the information above.
- Read your textbook and look carefully at the diagrams and photographs on the section concerning mitosis.
- Write down the key features that will enable you to identify root tip cells at each of the different stages of mitotic cell division.

### Method

#### Preparation

1. You have been provided with a germinated seedling of a broad bean or similar.
2. Using a sharp knife or scalpel, carefully cut off 5mm from the apical tip of five lateral roots.
3. Place the root tips into a test-tube containing acetic orcein stain.

Warning! Acetic orcein is an acidic stain that turns protein purple. Any stain that gets on skin or natural clothing will stain the material purple.

4. Warm the acetic orcein and root tips in a hot water bath but do NOT allow the solution to boil. The stain should be kept so that it is gently steaming for at least five minutes.
5. Using a mounted needle or similar, carefully remove one of the root tips. The root tips will now be very soft and easily damaged so do not remove them with forceps.
6. Leave the remaining four root tips in the hot stain.
7. Place the root tip onto a clean microscope slide.
8. Using a sharp knife cut it in half transversely so that each length is approximately 2.5mm.
9. Discard the half that is furthest away from the root tip.
10. Using a pipette, carefully add two or three drops of acetic orcein stain to the root tip on the slide.
11. Using a mounted needle or similar gently break the tissue of the root tip apart.
12. Carefully lower a cover slip over the root tip and using the blunt end of your mounted needle or a pencil, gently push the cover slip down onto the slide so that the root tip is squashed. Care is needed to ensure that as few air bubbles are present as possible.
13. Gently blot any excess stain from the slide.
14. Examine the slide using a microscope for the different stages in mitosis.
15. If insufficient staining has taken place, the process may be repeated with the other four root tips that have been left in the hot stain.

#### Making observations

1. Initially observe the tissue using the low power of your microscope. This will enable you to find areas of cells where the nuclear material is clearly visible and undergoing mitosis.

2. Using the high power of your microscope examine individual cells at various stages of division.
3. Using the high power of you microscope make large, labelled drawings of each of the stages of cell division.

Write-up

- Write up the method and answering the following questions
1. Suggest why the tissue was heated when placed in the acetic orcein stain.
  2. Explain how you placed the coverslip on the slide in order to reduce the number of air bubbles.
  3. What was the diploid number of chromosomes in the cells of the root tip that you examined?
  4. Explain why the haploid number is always even.

## Practical 8 - Lesson Plan

### Broad bean root tip squash

#### Context

A practical investigation set in the context of 9700 syllabus – Cell and nuclear division

#### Key aims of the lesson

This practical is designed to develop the skills of manipulation of apparatus and observation, and the recording and interpretation of observations.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods.
- Describe and explain the reasons behind the methods.
- Extend knowledge on the structure and function of mitosis

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – Student worksheet given out for students to read in preparation for the practical lesson. Students to read and look at drawings and photographs of mitosis in their textbooks.
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review the sequence of mitosis and determine the key features of each of the stages with key points written on board
5 - 10	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns when using hot acetic orcein stain
10 - 25	<b>Carrying out the practical</b> – students carry out the practical work.
25 - 50	<b>Obtain results</b> – Students observe mitosis and produce clear labelled diagrams of each stage, then clear away apparatus as soon as they have finished

50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the manipulation and observational skills that have been developed as well as discussion on results obtained. Practical write up to be completed in following lesson or as homework activity
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**Useful information**

- Other plant root tips can be substituted for broad bean, such as other types of bean, onions or sunflowers.
- The intensity of the stain depends upon how long the root tips are left in the hot acetic orcein stain.

Discussion / evaluation points should include:

- explanation of the methods used and how to successfully produce a mounted slide
- possible problems with the method such as the degree of staining, sufficient squashing of tissue and microscope technique

## Practical 8 - Technical information

### Broad bean root tip squash

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. 1 x actively growing broad bean seedling such that at least five lateral roots have developed. Broad bean may be substituted with any other bean, or onion or sunflower.
2. sharp knife or scalpel
3. 1 x test-tube and hot water bath that can be maintained at approximately 90°C
4. 1 x microscope slide and cover slip
5. 1 x mounted needle or similar
6. 5cm<sup>3</sup> of acetic orcein stain. If not previously prepared, the acetic orcein should be mixed with 1M HCl in the proportions of ten parts stain to one part acid.
7. Access to microscope with both low and high power objective lenses

#### Safety Precautions/Risks.

Acetic orcein = C



A risk assessment should be carried out as a matter of course.

#### **NOTE**

**Acetic orcein is an acidic stain that turns protein purple. Any stain that gets on skin or natural clothing will stain the material purple.**